***PPARG*, *GNG12*, and *CD19* are Potential Independent Predictors of Central Nerve Recurrence in Childhood Acute Lymphoblastic Leukemia**

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Research Article

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Abstract

Objective: To identify biomarkers that can predict the recurrence of the central nervous system (CNS) in children with acute lymphoblastic leukemia (ALL), and establish a prediction model. **Materials and**

Methods: The transcriptome and clinical data collected by the Children's Oncology Group (COG) collaboration group in the Phase II study (use for test group) and Phase I study (use for validation group) of ALL in children were downloaded from the TARGET database. Transcriptome data were analyzed by bioinformatics method to identify core (hub) genes and establish a risk assessment model. Univariate Cox analysis was performed on each clinical data, and multivariate Cox regression analysis was performed on the obtained results and risk score. The children ALL phase I samples collected by the COG collaboration group in the TARGET database were used for verification.

Results: A total of 1230 differentially expressed genes were screened out between the CNS relapsed and non-relapsed groups. Univariate multivariate Cox analysis of 10 hub genes identified showed that PPARG (HR=0.78, 95%CI=0.67-0.91, p=0.007), CD19 (HR=1.15, 95%CI=1.05-1.26, p=0.003) and GNG12 (HR=1.25, 95%CI=1.04-1.51, p=0.017) had statistical differences. The risk score was statistically significant in univariate (HR=3.06, 95%CI=1.30-7.19, p=0.011) and multivariate (HR=1.81, 95%CI=1.16-2.32, p=0.046) Cox regression analysis. The survival analysis results of the high and low-risk groups were different when the validation group was substituted into the model (p=0.018). In addition, the CNS involvement grading status at first diagnosis CNS3 vs. CNS1 (HR=5.74, 95%CI=2.01-16.4, p=0.001), T cell vs B cell (HR=1.63, 95% CI=1.06-2.49, p=0.026) were also statistically significant.

Conclusions: PPARG, GNG12, and CD19 may be predictors of CNS relapse in childhood ALL.

Introduction

Childhood acute lymphoblastic leukemia (ALL) is one of the most common primary malignant tumors in children and adolescents, accounting for about 35% of all tumors in children and more than 75% of all childhood acute leukemia cases^[1]. Although most ALL kids frequently achieve complete remission, about 3–8% of children with ALL suffer from relapse in the central nervous system (CNS). Those suffering from CNS leukemia (CNSL) are believed to have poorer outcomes than CNS-negative kids^[2, 3]. Traditional intrathecal injection of chemotherapeutic drugs and radiotherapy has been associated with side effects and CNS relapse after remission. In addition, the majority of CNS relapse occurs in patients who were CNS-negative at initial diagnosis^[4]. Therefore, exploring new favorable biomarkers for CNS relapse may significantly improve the early diagnosis and treatment in childhood ALL.

Studies have reported that gender, hepatomegaly, central nervous system status and age^[5], neuronal-glial antigen-2 (NG2) expression^[6], and CNS microenvironment^[7] are all associated with CNS relapse of childhood ALL. Another study showed that the up-regulation of the *PBX1* gene in B-cell leukemia in mouse CNS microenvironment could enhance the chemotherapy-resistance and self-renewal characteristics of leukemia cells^[8]. However, as these studies were based only on clinical data or animal experiments, there is still no independent predictor of CNS relapse in childhood ALL.

The aim of this study was to identify biomarkers that can predict the relapse of CNS in childhood ALL and establish a prediction model to provide early preventive therapy and targeted therapy for the childhood ALL at high risk of CNS relapse.

Materials And Methods

Clinical Data and preprocessing

We downloaded clinical and transcriptome data for Phase II of the ALL program from the TARGET database (<https://ocg.cancer.gov/programs/target>). The data obtained from the database were divided into CNS relapsed group and non-relapsed group based on the presence or absence of CNS relapse. The inclusion and exclusion criteria for the two groups of data were: (1) patients were younger than 18 years old at the time of diagnosis; (2) complete transcriptome data were obtained in both groups; (3) clinical information should include complete relapse-free survival, CNS relapse status, etc.; (4) relapsed group included patients that had only CNS relapse and no other site relapse at the end of the follow-up ;the non-relapsed group refers to patients with no recurrence in any part by the end of follow-up

Differentially expresses genes (DEGs) and enrichment analysis

The edge R was used to identify DEGs between the two groups (relapsed group vs. non-relapsed group). A false discovery rate (FDR) adjusted p < 0.05 combined with a simultaneous absolute value of ≥ 2 for logFC was set as the threshold for DEGs identification. The results of the differential analysis were visualized using gplots on the volcanic map and heat map. The differential genes were imported into the Biomart application of the Ensembl website for gene ID conversion. GO function enrichment analysis (annotated from the biological process (BP), cell composition (CC), molecular function (MF)), and KEGG pathway analysis was performed using the David website. The STRING was used for the analysis of protein interactions encoded by differential genes; the credibility >0.7 was used as a cut-off value to plot the Protein-protein interaction network (PPI).

Twelve topological analysis methods, including Degree Edge Percolated Component (EPC), Maximum neighbourhood component (MNC), Density of Maximum Neighborhood Component (DMNC), and Maximal Clique Centrality (MCC) etc, were provided by the CytoHubba plug-in in the Cytoscape software. These methods were used to analyze protein interaction networks. The top 10 scoring genes obtained by various algorithms were counted for frequency, and the top 10 genes with the highest occurrence frequency were regarded as hub genes.

Survival analysis and stepwise backward regression, ROC curve

The top 10 DEGs with the highest occurrence frequency were used as hub genes for univariate Cox regression analysis using Survival R packet. Multivariate Cox regression included statistically significant DEGs, and the stepwise backward regression was used to establish the optimal model. Finally, the risk value was calculated using the following formula: the risk score= (β_1 * expression level of *PPARG*) + (β_2 * expression level of *CD19*) + (β_3 * expression level of *GNG12*). The ROC curves of 3-5 years were drawn every six months in batches using the survival ROC package. ROC curve at the time point with the maximum AUC was selected to intercept the turning point, which was used as the cut-off value of the risk score. The research subjects were divided into high and low-risk groups based on the cut-off value of the risk score. The Kaplan-Meier survival curves for the cases predicted to have low or high risk were produced to verify whether the two groups showed statistical significance.

Validation

The data collected by the COG collaboration group in the TARGET database for phase I of the study on childhood ALL were used to verify. The expression levels of the three target genes identified in the study were included in the model, and the risk score was calculated. The high-risk group and the low-risk group were also divided, and survival analysis was conducted to verify the accuracy of the model. Finally, to further validate whether the biomarkers' prediction was independent of other clinical variables, univariate and multivariate Cox regression, stratified analyses were conducted.

Statistical and analytical software

R3.6.0 was used for all data analysis and mapping. Gene ID conversion through Ensembl (<https://grch37.ensembl.org/info/data/biomart/index.html>). Enrichment analysis was conducted by the David website (<https://david.ncifcrf.gov/tools.jsp>). PPI plot was drawn by String website String (<https://string-db.org/>). Hub gene was determined by Cytoscape 3.8.2. P-value of < 0.05 was considered as statistical significance, and all reported p-values were two-sided.

Results

Patient characteristics

Clinical information of the subjects in the relapsed group and non-relapsed group were described by means and percentages. A total of 278 subjects were included in this study, with an average age of 8.7±4.6 years. The time of diagnosis was from 2001 to 2011, and the follow-up period was from 2005 to 2016(the mean follow-up time was 6.3 years); the male to female ratio was 3:7. Based on the presence or absence of CNS relapse, the data were divided into CNS relapsed and non-relapsed groups, with 28 patients in the CNS relapsed group and 250 patients in the non-relapsed group. Due to many missing values of the *ETV6-RUNX1* fusion gene and only 4 positive cases, and since only one case of *TCF3-PBX1* and *BCR-ABL1* genes were positive, subsequent studies were not included in the analysis (**Table 1**).

DEGs analysis

A total of 1230 differential genes were screened, including 685 up-regulated and 545 down-regulated genes. G plots were used to visualize the different analysis results, as shown in **Figure 1** and **Figure 2**.

Identification of Differentially Expressed Genes and Functional Enrichment Analysis

The results showed that these differential genes were mainly involved in biological processes such as antigen processing and positive regulation of T cell aggregation, angiogenesis, and cell adhesion, migration, and proliferation of presenting cells. The cell components were mainly concentrated on cell surface, transport vesicle membrane, endocytic vesicle membrane, and involved in the composition of the MHC class II protein complex. As for molecular function, the results showed that genes were mainly involved in MHC class II receptor activity, growth factor activity, signal transduction activity, calmodulin-binding activity, etc. ($p < 0.05$) (**Figure 3A**). KEGG pathway analysis showed that these genes were mainly involved in antigen processing and presentation-related pathways, PI3K-Akt signaling pathway, Ras signaling pathway, and cancer-related signaling pathways ($p < 0.05$) (**Figure 3B**).

Protein interaction network (PPI) and hub gene identification

A total of 580 proteins and 647 edges were obtained with a cut-off value of credibility > 0.7. The average interaction between each node was 2.23, and the aggregation coefficient was 0.315, as shown in **Figure 4**.

Frequency statistics were carried out for the top 10 genes scored by various algorithms, and the top 10 genes (*NPY*, *IL1B*, *IL6*, *BMP2*, *PPARG*, *CD19*, *GNG13*, *CD40*, *GNG7*, *GNG12*) with the highest occurrence frequency were regarded as hub genes (**Table 2** and **Figure 5**).

PPARG, *CD19*, and *GNG12* were obtained by Cox regression analysis

Univariate Cox regression analysis was performed for the top 10 hub genes using Survival packet (recurrence as the outcome variable, time without recurrence as time-variable). The results showed that all the 10 hub genes were statistically significant, and they were included in multivariate Cox regression analysis (**Table 3**).

The results of multivariate analysis showed that there were three genes with significant differences, namely *PPARG*, *CD19*, and *GNG12* (**Table 3**). In the CNS relapsed group, *PPARG* showed the lowest expression, while *CD19* and *GNG12* had the highest expression. The risk model was obtained according to the regression results as follows:

$$\text{risk score} = PPARG * (-0.19009) + CD19 * (0.13794) + GNG12 * (0.22447)$$

For the obtained risk model, the ROC curves at multiple time points across 3-5 years were done in batches to compare and screen out the time point at which the maximum AUC was obtained. The results showed that the model established in this study has a good predictive ability in predicting the 5-year relapse rate (AUC max=0.75) (**Figure 6**).

We calculated the risk score of the 278 research objects. The true positive and false positive difference maximum points were taken as cut-off of the risk score. Values above this score were defined as high-risk group, and below this score as a low-risk group. Kaplan–Meier survival curve and log-rank test were adopted to appraise the association of the overall survival (**Figure 7**).

In order to verify the accuracy of the model, we used transcriptome and clinical data collected from the COG collaboration group in the Target database for the phase of childhood ALL for verification. The expression levels of the three target genes identified in the study were included in the model, and the risk values were calculated. The risk groups were also divided into high and low-risk groups, and survival analysis (K-M curve) was performed. Our results proved that the established model has a good predictive ability ($p=0.018$) (**Figure 8**).

The risk score can be used as an independent prognostic factor

To judge whether the model's risk score can be used as an independent predictor of a childhood ALL relapse of CNS, we conducted Cox regression analyses with clinical data (**Table 4**). The results showed that the risk score was statistically significant in univariate (HR=3.06, 95%CI=1.30-7.19, $p=0.011$) and multivariate (HR=1.81, 95%CI=1.16-2.32, $p=0.046$) Cox regression analysis. CNS3 vs. CNS1 (HR=5.74, 95%CI=2.01-16.4, $p=0.001$) and T cell vs. B cell (HR=1.63, 95%CI=1.06-2.49, $p=0.026$) were significantly different between the two groups; while no difference was found for patients' gender (HR=1.20, 95%CI=0.72-2.02, $p=0.490$).

We stratified the expression of the *CD19* gene according to the source of T cells and B cells. The results showed that this gene differed only in B lymphocytic leukemia ($p=0.018$), as shown in **Table 5**.

Discussion

The CNS is a common site for relapse of ALL. Yet, considering that most drugs cannot pass through the brain blood-brain barrier (BBB), the treatment of CNS leukemia is still very challenging^[9]. Therefore, identifying new potential biomarkers that could predict CNS relapse in children is extremely important. In this study, bioinformatics correlation analysis was used to identify biomarkers, revealing that *PPARG*, *GNG12*, and *CD19* were independent prognostic factors for childhood ALL with CNS relapse.

PPARG gene is a member of the nuclear receptor family, which encodes peroxisome proliferative activated receptor gamma (PPAR γ), PPAR γ was first identified in adipocytes, as a transcription factor, the PPAR γ receptor binds to its ligand and inhibits tumor proliferation, metastasis, angiogenesis, and promotes apoptosis and immune regulation by activating signaling pathways of different tumor cells and tumor stem cells^[10]. For example, PPAR γ can induce apoptosis by inhibiting the PI3K/Akt signaling pathway, PPAR γ ligand can induce the apoptosis of leukemia cells, and can be potentially used to treat hematologic malignancies, especially leukemia^[11]. In our study, we found a low expression of *PPARG* in childhood ALL with CNS relapse. Moreover, KEGG analysis showed activation of the PI3K/Akt signaling pathway, which suggests that *PPARG* might regulate CNS relapse in ALL children through the PI3K/Akt signaling pathway. Boyd *et al*^[12] found that the injection of PPARs antibody agonist could induce adipogenesis in bone marrow tissue in mice, thereby saving healthy hematopoietic cell formation during the inhibition of leukemia cell growth. The most common way leukemia cells invade the central nervous system is from the bone marrow of the skull to the subarachnoid space via dural bridging vein^[13]. When the expression of the *PPARG* gene is low, this induction function of PPARs is inhibited, which might in turn lead to the CNS relapse of ALL in children.

The *GNG12*, a gene encoding G γ 12, a member of the G12 subfamily of heterotrimeric G proteins, is involved in the regulation of expression and distribution of tightly connected endothelial cells^[14]. The BBB is mainly composed of tight junctions (TJ) found between endothelial cells, which limit the passage of substances in the brain^[15]. Donato *et al* found that heterotrimer G protein has an important role in TJ's formation and regulation, which is regulated through the intercellular signaling pathway and has an important influence on the BBB structure^[16]. It has also been found that G γ 12 preferentially interacts with adrenergic receptors in activated central synapses^[17]. Adrenergic receptors are involved in the regulation of protein kinase A (PKA) and cAMP^[18]. The BBB appears to be highly sensitive to PKA levels and opens when cAMP and PKA levels decrease^[19]. This may result in leukemic cells crossing the blood-brain barrier more easily and invading the CNS.

CD19 play a key role in maintaining the balance of humoral immunity, antigen induction, and immune tolerance. The relationship between *CD19* and ALL has not been fully defined. Still, it is well known that CD19 is the most densely expressed antigen on B cells' surface in ALL patients and is a highly specific target for drug development^[20]. In particular, CAR T cells can penetrate the BBB into the CNS, killing leukemic cells in the cerebrospinal fluid^[21]. Jin MY *et al*^[22] reported two cases of acute leukemia patients with CNSL, which showed positive CD19 expression in tumor cell immunotyping and remission after anti-CD19 CAR T cell therapy. In addition, studies have reported the efficacy of anti-CD19 CAR T cell therapy in patients with ALL-CNSL^[23-26]. These treatments further revealed the relationship between *CD19* and CNSL, and also verified the high relapse risk of CNS in patients with high expression of the *CD19* gene in this study. Meanwhile, stratified analysis results show that this difference only appeared in B-ALL, which also verified the reliability of the results of this study. In other words, the high expression of the *CD19* gene is not only a marker of B-ALL but may also lead to CNS relapse when its expression level reaches a certain level.

In this study, the cell composition, molecular function, biological process, and KEGG pathway corresponding to the DEGs were enriched and analyzed. These results revealed a related pathway leading to the relapse of CNS, in which calmodulin-binding was related to the molecular composition and signal regulation

of the tight junction of the BBB. Some researchers have reported that leukemia cells enter CNS via vascular transport, which involves the interaction between A6 integrin and laminin and can be blocked by specific PI3K inhibitors^[27]. Our results further suggest that CNS relapse in childhood ALL is related to the PI3K-Akt signaling pathway. In addition, cell adhesion molecules (CAMs), antigen processing and presentation, and neuroactive ligand-receptor interactions provide upstream and downstream factors and targets for future studies on CNSL recurrence, which should be further explored.

The results of our study confirmed that CNS grade 3 at initial diagnosis was a risk factor for CNS relapse, which was consistent with previous studies^[6]. Our data also suggested that T lymphocytic leukemia in children was more likely to lead to CNS relapse than B lymphocytic leukemia, which was also consistent with previous relevant reports^[28-30]. This study failed to prove that hyperleukocytosis and male in patients were high-risk factors for CNS relapse, which were inconsistent with previous studies^[5,31]. We speculate that it may be due to sample differences and should be further clarified by future studies.

To sum up, this study verified that CNS3 classification and the types of T lymphocytes are risk factors for CNS relapse of childhood ALL. We found that *PPARG*, *GNG12* and *CD19* can be used as independent predictors of CNS relapse in childhood ALL, yet, more studies are needed to confirm these findings. Our results may provide a theoretical basis for further studies to elucidate the molecular mechanism of CNS relapse in childhood ALL and provide more therapeutic targets for future clinical interventions.

Declarations

Author's Contribution—Shan Zhang, Yansong Tu, Qianmiao Wu, Huijun Chen, Huaijun Tu and Jian Li. wrote the main manuscript text and prepared figures 1-8 and tables 1-5. All authors reviewed the manuscript.

Ethics and dissemination: This study does not need to be reviewed by the Ethics Committee, because this paper is not a clinical study or a related experimental study, and this paper is only a literature study.

Availability of Data and Materials : Our study received written approval from the NCI Office of Cancer Genomics. The results published here are in whole based upon data generated by the Therapeutically Applicable Research to Generate Effective Treatments (<https://ocg.cancer.gov/programs/target>) initiative, phs000218. The data used for this analysis are available at <https://portal.gdc.cancer.gov/projects>. We acknowledge the TARGET database for providing their platforms and contributors for uploading their meaningful datasets.

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Conflict of interest—We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Tables

Table 1. Patients’ characteristics

Term	total (n=278)	relapsed (n=28)	non-relapsed (n=250)
Age (years) mean±SD	8.7±4.6	7.4±5.0	8.8±4.5
Gender (n, %)			
Male	84 (30.2)	13 (46.4)	71 (28.4)
Female	194 (69.8)	15 (53.6)	179 (71.6)
Race (n, %)			
White	205 (73.7)	24 (85.7)	181 (72.4)
Others ^a	73 (26.3)	4 (14.3)	69 (27.6)
Race ^b (n, %)			
Hispanic or Latino	49 (18.9)	8 (28.6)	41 (17.7)
Not	210 (81.1)	20 (71.4)	190 (82.3)
Relapse-free survival (months)	76.9±26.4	60.6±32.1	78.7±25.1
Survival status (n, %)			
Alive	261 (93.9)	11 (39.3)	250 (100)
Dead	17 (6.1)	17 (60.7)	0 (0)
WBC count (10 ⁹ /L) (n, %)			
>100	128 (46.0)	12 (42.9)	128 (46.0)
(10,100]	124 (44.6)	13(46.4)	124 (44.6)
≤10	26 (9.4)	3 (10.7)	26 (9.4)
Day-29 MRD	0.51±1.3	0.51±1.3	0.45±1.3
Day-29 MRD ^b (n, %)			
>0	98 (35.5)	12 (42.9)	86 (34.7)
0	178 (64.5)	16 (57.1)	162 (65.3)
CNS classification ^b (n, %)			
CNS1	197 (71.1)	18 (64.2)	179 (71.9)
CNS2	61 (22.0)	5 (17.9)	56 (22.5)
CNS3	19 (6.9)	5 (17.9)	14 (5.6)
ETV6 RUNX1 ^b (n, %)			
Negative	122 (96.8)	16 (84.2)	106 (99.1)
Positive	4 (3.2)	3 (15.8)	1 (0.9)
TCF3-PBX1 ^b (n, %)			
Negative	199 (99.5)	18 (94.7)	181 (100)
Positive	1 (0.5)	1 (5.3)	0 (0)
BCR-ABL1(n, %)			
Negative	277 (99.6)	28 (100)	249 (99.6)
Positive	1 (0.4)	0 (0)	1 (0.4)
TRISOMY 4 10 Status ^b			
Negative	221 (96.5)	23 (100)	198 (96.1)
Positive	8 (3.5)	0	8 (3.9)
MLL Status ^b (n, %)			
Negative	227 (95.4)	26 (100)	201 (94.8)

Positive	11 (4.6)	0	11 (5.2)
BMA Blasts Day 8	20.3±26.3	30.0±31.4	19.2±25.6
BMA Blasts Day 15	4.6±8.8	7.4±16.8	4.3±7.1
BMA Blasts Day 29	0.7±1.2	1.1±1.5	0.7±1.1
DNA Index ^b (n, %)			
1	228 (83.8)	20 (76.9)	208 (84.6)
>1	44 (16.2)	6 (23.1)	38 (15.4)
Cell of origin ^b (n, %)			
B Cell	74 (27.1)	13 (50.0)	61 (24.7)
T Cell	199 (72.9)	13 (50.0)	186 (75.3)

Table 2. Twelve Topological analysis methods of top 10 genes ranked by Degree

MCC	DMNC	MNC	Degree	EPC	BottleNeck	EcCentricity	Closeness	Radiality	Betweenness	Stress	ClusteringCoefficient
<i>GNG13</i>	<i>HLA-DMA</i>	<i>IL6</i>	<i>IL6</i>	<i>IL6</i>	<i>IL6</i>	<i>IL6</i>	<i>IL6</i>	<i>IL6</i>	<i>IL6</i>	<i>IL6</i>	<i>SYT13</i>
<i>GNG7</i>	<i>HLA-DOA</i>	<i>GNG13</i>	<i>GNG13</i>	<i>CD19</i>	<i>IL1B</i>	<i>IL1B</i>	<i>IL1B</i>	<i>IL1B</i>	<i>NPY</i>	<i>NPY</i>	<i>LOXHD1</i>
<i>GNG12</i>	<i>F2RL3</i>	<i>GNG7</i>	<i>GNG7</i>	<i>IL1B</i>	<i>NRP1</i>	<i>NPY</i>	<i>BMP2</i>	<i>NPY</i>	<i>NRP1</i>	<i>GNG13</i>	<i>HLA-DMA</i>
<i>GNG11</i>	<i>CYSLTR2</i>	<i>CD19</i>	<i>CD19</i>	<i>CD40</i>	<i>PPARG</i>	<i>CCL4L1</i>	<i>NPY</i>	<i>BMP2</i>	<i>CD19</i>	<i>GNG7</i>	<i>LDOC1</i>
<i>BDKRB2</i>	<i>EDNRB</i>	<i>GNG12</i>	<i>CD40</i>	<i>IRF4</i>	<i>NPY</i>	<i>PPARG</i>	<i>CD19</i>	<i>PPARG</i>	<i>IL1B</i>	<i>PRKCG</i>	<i>HLA-DOA</i>
<i>BDKRB1</i>	<i>UTS2R</i>	<i>CD40</i>	<i>GNG12</i>	<i>GNG13</i>	<i>GNG13</i>	<i>HPGDS</i>	<i>ENG</i>	<i>ENG</i>	<i>PRKCG</i>	<i>IL1B</i>	<i>CLIC5</i>
<i>NPY</i>	<i>HLA-DRB5</i>	<i>GNG11</i>	<i>IRF4</i>	<i>NPY</i>	<i>GRID2</i>	<i>GJA3</i>	<i>PPARG</i>	<i>CD19</i>	<i>HTR5A</i>	<i>HTR5A</i>	<i>AFAP1L1</i>
<i>SUCNR1</i>	<i>HLA-DPB1</i>	<i>IRF4</i>	<i>BMP2</i>	<i>GNG12</i>	<i>GRIA2</i>	<i>CYGB</i>	<i>CD40</i>	<i>GJA1</i>	<i>GRIA2</i>	<i>CD19</i>	<i>TMEM88B</i>
<i>HTR5A</i>	<i>SUCNR1</i>	<i>VWF</i>	<i>IL1B</i>	<i>GNG7</i>	<i>KLF4</i>	<i>KLF2</i>	<i>GJA1</i>	<i>CTGF</i>	<i>PPARG</i>	<i>PPARG</i>	<i>HS6ST2</i>
<i>SSTR4</i>	<i>NPFFR2</i>	<i>BMP2</i>	<i>NPY</i>	<i>GNG11</i>	<i>PRKCG</i>	<i>STK32B</i>	<i>CTGF</i>	<i>CD40</i>	<i>BMP2</i>	<i>NRP1</i>	<i>CYSLTR2</i>

Table 3. Cox regression results of hub gene

gene	Univariate cox.			Multivariable Cox.		
	β	HR(95%CI)	p-value	β	HR(95%CI)	p-value
<i>NPY</i>	0.17	1.18(1.10-1.28)	<0.01			
<i>IL1B</i>	0.21	1.23(1.10-1.39)	<0.01			
<i>IL6</i>	0.26	1.29(1.07-1.56)	<0.01			
<i>BMP2</i>	0.18	1.20(1.12-1.28)	<0.01			
<i>PPARG</i>	-0.21	0.81(0.66-0.93)	0.039	-0.19	0.78(0.67-0.91)	0.007
<i>CD19</i>	0.19	1.21(1.11-1.31)	<0.01	0.14	1.15(1.05-1.26)	0.003
<i>GNG13</i>	0.29	1.34(1.07-1.68)	0.012			
<i>CD40</i>	0.30	1.35(1.17-1.55)	<0.01			
<i>GNG7</i>	0.19	1.21(1.10-1.32)	<0.01			
<i>GNG12</i>	0.35	1.42(1.19-1.70)	<0.01	0.23	1.25(1.04-1.51)	0.017

Table 4. Cox regression results of independent predictors

	Univariate Cox		Multivariate Cox	
	Relapse-free survival		Relapse-free survival	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age (0,6]	1			
(6,12]	1.72 (0.66-4.48)	0.265		
(12,18]	0.91 (0.32-2.63)	0.863		
Sex (female vs male)	2.10 (1.09-4.42)	0.017	1.20 (0.72-2.02)	0.490
Race (others ^a vs White)	0.34 (0.05-2.51)	0.289		
Eth (Hispanic vs Not)	1.70 (1.02-2.63)	0.204		
WBC count $\times 10^9/L$				
≤ 10	1			
(10,100]	1.19 (0.34-4.25)	0.780		
>100	1.11 (0.51-2.42)	0.803		
Day-29 MRD (>0 vs 0)	1.70 (1.13-2.57)	0.360		
CNS classification				
CNS1	1			
CNS2	0.86 (0.32-2.32)	0.770	0.52 (0.19-2.29)	0.519
CNS3	3.38 (1.25-9.10)	0.016	5.75 (2.01-16.4)	0.001
Trisomy (N v P)	0.98 (0.36-2.67)	0.964		
MLL (N v P)	3.57 (0.50-25.66)	0.206		
BMA Blasts Day 8 (≥ 6 vs ≤ 6)	1.17 (0.79-1.71)	0.436		
BMA Blasts Day 15 (≥ 2 vs < 2)	1.06 (0.64-1.76)	0.829		
BMA Blasts Day 29 (>0)	0.98 (0.64-1.50)	0.930		
DNA index > 1 vs ≤ 1	1.50 (0.61-3.73)	0.386		
T Cell V B Cell ALL	2.59 (1.18-5.68)	0.017	1.63 (1.06-2.49)	0.026
Prediction model Risk score	3.06 (1.30-7.19)	0.011	1.81 (1.16-2.32)	0.046

a, Include black and Native Hawaiian or other Pacific Islander Asian and Unknown;

Table 5. Layered analysis of *CD19* gene between T cells and B cells

Non-relapse vs. relapse	Log FC	P-value	FDR
B-cells	2.176	0.018	0.037
T-cells	-0.318	0.184	0.367

Figures

Figure 1

Volcano map of DEGs a yellow represents up-regulated genes, blue represents down-regulated genes, and black represents no statistically significant genetic difference.

Figure 2

Heat map of DEGs

Figure 3

Gene ontology (A) and KEGG (B) pathway analysis of DEGs The top 10 pathway with the smallest p value were selected for plotting. GO, Gene Ontology; BP, biological process; CC, cell composition; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes;

Figure 4

Differential gene-protein interaction networks. Cut-off value of credibility > 0.7, received 580 proteins and 647 edges.

Figure 5

Top 10 genes with frequency. Top 10 genes with the highest occurrence frequency of twelve topological analysis methods were used for frequency statistics.

Figure 6

ROC curve of the prediction model. ROC curves at multiple time points across 3-5 years were done in batches to compare and screen out the time point at which the maximum AUC was obtained.

Figure 7

Kaplan-Meier curve of high risk and low-risk group. The true positive and false positive difference maximum points were taken as cut-off of the risk score. Values above this score were defined as a high-risk group, and below this score as a low-risk group

Figure 8

Kaplan-Meier curve of high and low risk of the validation group. The true positive and false positive difference maximum points were taken as cut-off of the risk score. Values above this score were defined as a high-risk group, and below this score as a low-risk group